Direct Nanolayer Preparation of Molecularly Imprinted Polymers Immobilized on Multiwalled Carbon Nanotubes as a Surface-Recognition Sites and Their Characterization

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ABSTRACT: Molecular imprinting is a method for making artificial receptor sites in a polymer. This article reports the direct nanolayer immobilization of molecularly imprinted polymers (MIPs) on hydroxyl-functionalized multiwalled carbon nanotubes (MWCNTs) without any binder to improve their characteristics. MIPs were formed for hydrochlorothiazide (HCT) as a template on the surface of the MWCNTs with methacrylic acid (functional monomer) and ethylene glycol dimethacrylate (crosslinking agent) with a thermal polymerization technique. The morphology and stability of the immobilized molecularly imprinted polymers on the surface of multiwalled carbon nanotubes (MIPCNTs) was characterized with scanning electron microscopy, Fourier transform infrared spectroscopy, and thermogravimetric analysis. The resulting MIPCNTs demonstrated favorable selectivity, good stability, and a higher adsorption capacity for the template molecule (93.0 μg/mg) compared to products created by bulk polymerization. The adsorption kinetics of HCT at the surface of the MIPCNTs was in agreement with the second-order rate equation. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 798–803, 2012

Key words: molecular imprinting; molecular recognition; nanolayers

INTRODUCTION

Molecularly imprinted polymers (MIPs) are especial polymers that are attained by the polymerization of a monomer with a crosslinker in the presence of a template molecule. Molecular recognition sites are formed after the extraction of the template molecule from the polymerized material. The formed cavities are complementary to the template in size and shape for the subsequent rebinding process. Therefore, the MIPs are selectively able to interact with the analyte from a complex matrix, which endows them with special characteristics.

Recently, MIPs have been used in miscellaneous applications because of their molecular identification ability, steady state under various circumstances, and high stability against organic solvents.[1,2] Some MIPs have been used to identify olive oil,[3] drugs,[4,5] small analytes,[6] peptides[7] and proteins.[8–11] The preparation of MIPs is easy and is easily coupled with other detection systems, such as gas chromatography,[12] high-performance liquid chromatography (HPLC),[13] and ion mobility spectrometry.[14] However, some limitations, such as a heterogeneous distribution of binding sites in the network polymer, poor site accessibility for template molecules, and slow kinetic binding times, are endured with application of the MIPs. Also, the main drawback of MIP applications in electrochemical techniques is a lower conductivity.

Nowadays, multiwalled carbon nanotubes (MWCNTs) are considered for their high electrical and thermal conductivity properties.[15] Because of their unique characteristics in variety of applications, MWCNTs have successfully been used to detect proteins, tumor markers, and some drugs.[16,17] Also, MWCNTs can be an outstanding option as a supported material to overcome the previously discussed problems that are encountered with use of the MIPs. Through the formation of the MIP on the surface of MWCNTs, the accessibility of the analyte to binding sites can be improved, and the binding time can be reduced.

In an effort to improve MIP properties, they can be immobilized as nanolayer recognition sites on MWCNTs. The main aim of this work was the direct nanolayer preparation and characterization of molecularly imprinted polymers on multiwalled carbon nanotubes (MIPCNTs) for hydrochlorothiazide (HCT) as a template that could exhibit better molecular-recognition properties. Techniques such as scanning electron microscopy (SEM), Fourier transform

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infrared (FTIR) spectroscopy, and thermogravimetric analysis (TGA) were used to confirm the MIPCNT distinctiveness.

EXPERIMENTAL

Materials

HCT, phenazopyridine (PAP), and primidone (PM) were supplied from Sigma-Aldrich (Monich, Germany). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), 2,2′-azobis(2-isobutyronitrile) (AIBN), dimethylformamide (DMF), HPLC-grade methanol, and acetic acid were purchased from Merck (Honenbrunn, Germany). MWCNTs were brought from Iran's Research Institute of Petroleum Industry and were synthesized by chemical vapor deposition with a purity of 95%.

Apparatus

The polymerized surfaces and morphology of the MIPCNTs were observed by SEM (XL30, Philips, Amsterdam, Netherlands) and field emission SEM (Hitachi, S-4160, Japan). TGA (BAHR Thermoanalyse, STA 503) was performed under N_2 with purging from 20 to 700°C at a heating rate of 20°C/min. An ultraviolet–visible (UV–vis)/near infrared spectrophotometer (Jasco, V-570) (Tokyo, Japan) was used to record the absorption spectra. FTIR spectra were recorded (Jasco, FTIR-680 Plus) and used to examine the chemical bonding. To separate the MIPCNTs or immobilized nonimprinted polymers on the surface of multiwalled carbon nanotubes (NIPCNTs) from the suspension solution, centrifugation (Hermle Labortechnik GmbH, model Z 36 HK) was used at 30,000 rpm for 10 min.

Immobilization of the polymer on the MWCNTs

For the formation of hydrophilic groups, such as carboxyl groups, on carbon nanotubes, different methods have been reported in the literature. Therefore, on the basis of the oxidation ability of HNO_3 to form carboxylic groups on the surface of MWCNTs, it was used as an oxidizing agent.[18] The MWCNTs containing carboxylic functional groups were prepared as follows: 500 mg of crude MWCNTs was added to a glass reactor containing 60 mL of HNO_3, and the mixture was kept under ultrasonication for 15 min. This mixture was refluxed for 22 h at 80°C and then cooled, filtered, and washed by passage through about 5 L of distilled water.

The modified MWCNTs were used as a support material for the formation of polymer on the surface. Amounts of 0.1678 g (1.95 mmol) of MAA, 1.9325 g (9.76 mmol) of EDMA, and 4.25 mL of DMF were thoroughly mixed with 0.118 g (0.396 mmol) of HCT as a template molecule, and then, 0.025 g of AIBN was added to this solution. The molar ratio of the template to monomer to crosslinker was 1 : 5 : 25. After that, 50.0 mg of modified MWCNTs was added under ultrasonication for 15 min. The temperature and time of polymerization in the process were 25°C and about 3 days, respectively. The resulting MWCNT particles, which were decorated with polymer, were separated from the mixture and dried at room temperature overnight. HCT was removed from the polymer matrix by four washings with 10 mL of a methanol–acetic acid solution (9 : 1) for 6 h and two washings with water. With the formation of polymer on the MWCNT surface, template removal was easy and did not require harsh conditions. Nonimprinted polymer (NIP) was also prepared in the same way without the addition of HCT as a reference.

Adsorption experiments of the MIPCNTs and NIPCNTs

To monitor the HCT, PAP, and PM adsorption on the MIPCNTs or NIPCNTs, a UV–vis spectrophotometer was used as a simple and suitable apparatus. The rebinding experiment was carried out by the incubation of 10.0 mg of MIPCNTs or NIPCNTs in 10-mL HCT, PAP, and PM solutions (20 μg/mL). After 10 min of centrifugation at 30,000 rpm, 1.0 mL of supernatant was transferred into the UV–vis spectrophotometer cell to measure the absorbances of HCT, PAP, and PM at 270, 430, and 255 nm, respectively. The binding capacity (A) was defined as the number of micrograms of template bound per milligrams of presented MIPs or NIPs on the MWCNTs. A was measured on the basis of the differences in the HCT, PAP, and PM concentrations before and after adsorption in a constant volume of aqueous solution and the specified weight of the MIPCNTs or NIPCNTs according to Eq. (1):

$$A = (C_0 - C)V/M \quad (1)$$

where C_0 and C are the initial concentration (μg/mL) and the concentration after adsorption on the MIPCNTs or NIPCNTs, respectively, V is the volume of HCT solution (mL), and M is the weight of the MIPs or NIPs (mg) that were immobilized on the MWCNT.

Adsorption kinetics

The adsorption kinetics of HCT on the imprinted polymer were determined in a batch process. An amount of 10.0 mg of MIPCNT was placed in 10.0 mL of a 30.0 μg/mL HCT solution. Afterward, 0.05 mL of this solution at different time intervals was added to 1.0 mL of water. The mixture was centrifuged, and

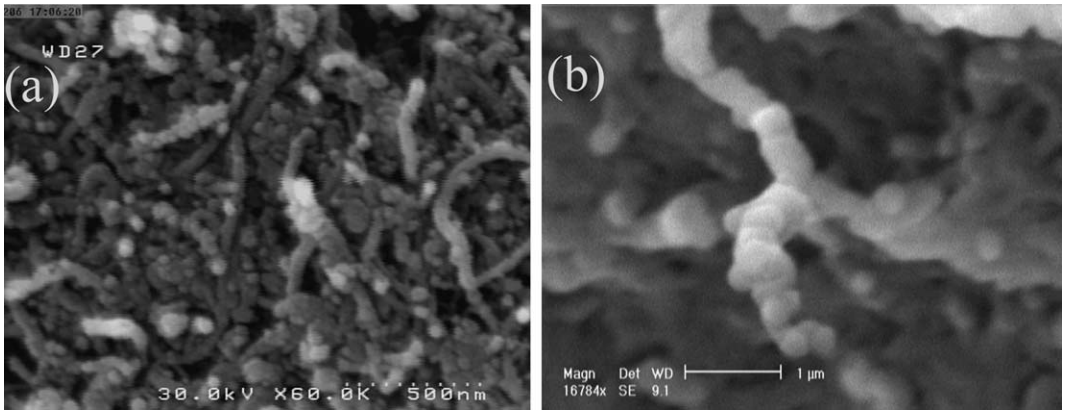

Figure 1 Morphological structure of the (a) MWCNTs and (b) MIPCNT particles.

the concentrations of HCT were measured at 270 nm with a UV–vis spectrophotometer. The adsorption amount was calculated with Eq. (1).

RESULTS AND DISCUSSION

SEM images of the MWCNTs and MIPCNTs

The morphological structure of the MWCNT particles was observed with SEM, as shown in Figure 1(a). The average thickness of the wall and the length of the MWCNTs were about 40 nm and several micrometers, respectively. With the immobilization of the polymer on the surface of the MWCNTs, the thickness was increased about 250 nm, as shown in Figure 1(b). This result could be used as a confirmation of the successful formation of MIPCNTs and revealed that the MIP layer was successfully attached to the MWCNT surface. Furthermore, the surface of the MIPCNTs was rougher than that of the MWCNTs; this suggested that a layer of MIPs covered the MWCNTs without changing the morphology of the MWCNTs.

Effects of the monomer and crosslinker concentrations

To access a good recognition binding site and compare the thickness of the MIP layer that was deposited on the surface of the MWCNTs, the amounts of monomer and crosslinker were varied. Table I shows the amounts of MAA and EDMA that were taken. The SEM technique was applied to observe the variations in size of the prepared MIPCNTs. As can be seen in Figure 2, with decreasing amounts of MAA and EDMA, the thickness of the MIP layer was reduced (ca. 70 nm), where in this case, the polymer on the surface of the MWCNTs acted as a better access for the template to the binding sites.[19]

FTIR spectra of the MIPCNTs

The MWCNTs, MIPs, and MIPCNTs were characterized by FTIR spectroscopy, as shown in Figure 3. The $-CH_3$ and $=CH_2$ vibrations at 2931 and 2864 cm^{-1} obviously appeared at the spectrum of the MWCNTs processed with the acidic solution [Fig. 3(3)]. The strong peaks [Fig. 3(1–3)] at 1673 cm^{-1} were attributed to the carbonyl group. The main adsorption peaks of the MIPCNTs [Fig. 3(2)] situated around 3467, 1256, and 1155 cm^{-1} were assigned to the O—H stretching vibrations and C—O stretching vibrations of symmetric and asymmetric esters, respectively. Furthermore, additional peaks at 1296, 1321, and 946 cm^{-1} could be found in the spectrum [Fig. 3(2)] and were the obvious reason for the formation of polymer on the surface of the MWCNTs.

Adsorption isotherms of the MIPCNT and NIPCNT particles

It seemed that the significant forces for interaction between the MIPCNTs and NIPCNTs with HCT were the hydrogen bonding and hydrophilic forces. These forces, associated with cavity conformations, were the main affinity and specificity of MIPs to the template. To compare the capacity of adsorption bulk polymerization and formation of MIPs at the

TABLE I
Effect of the Monomer and Crosslinker Concentrations on the Thickness Layer of the MIPs

Polymer	MAA (g)	EDMA (g)	DMF (mL)	AIBN (g)	MWCNT (g)
MIPCNT 1	0.1678	1.9325	4.25	0.0250	0.0500
MIPCNT 2	0.0839	0.6441	4.25	0.0125	0.0500

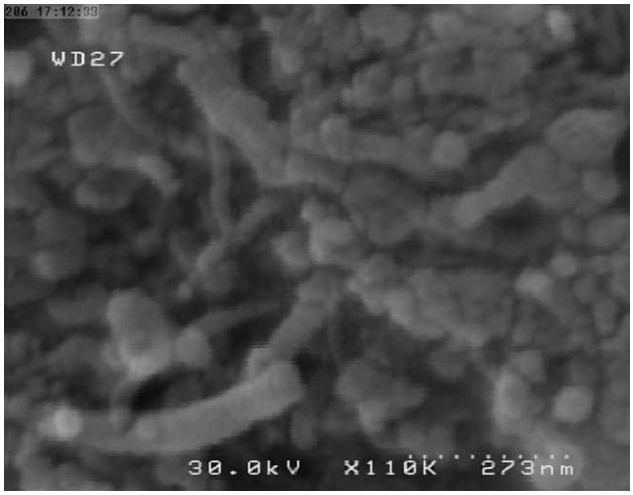

Figure 2 Effects of the monomer and crosslinker concentrations on the thickness of the MIP layer.

surface of the nanomaterials (MWCNTs), HCT was chosen as a template. The adsorption isotherms of HCT are represented in Figure 4. The adsorption capacity of the MIPCNTs (93.0 μg/mg) was much higher than that of the NIPCNTs. The imprinting efficiency was 8.1; this was obtained by the proportion of the maximum adsorption quantity of the MIPCNTs to that of the NIPCNTs. In addition, the capacity of the polymer in the MIPCNTs was higher than that of the polymer in bulk polymerization.[20] This revealed that the imprinted layer had a recognition ability for HCT, which could be ascribed to the formation of complementary cavities.

TGA

Because of the different thermal stabilities between the bare MWCNTs and MIPCNTs, thermogravimetric thermograms were used to determine the content of formed MIPs on the surface of the MWCNTs. Figure 5 shows

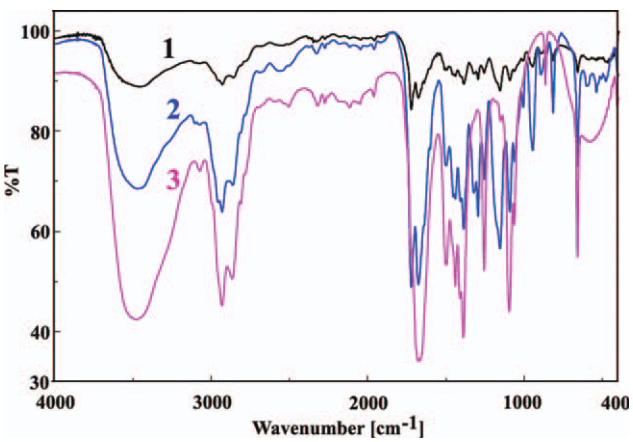

Figure 3 FTIR spectra of the (1) MIP, (2) MIPCNT, and (3) MWCNTs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

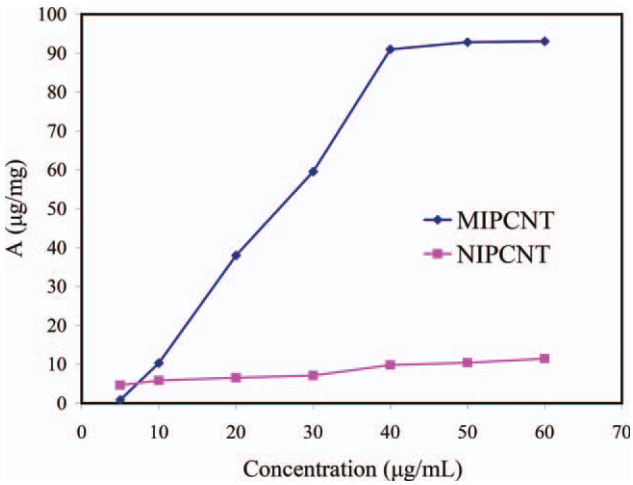

Figure 4 Adsorption isotherms of the MIPCNT and NIPCNT particles for HCT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the TGA weight losses of the MWCNTs, MIPCNTs, and MIPs. The results show that the MWCNTs were stable, and we did not observe loss weight below 700°C. However, the MIPCNTs were steady to 277°C, but with increasing temperature to 470°C, the immobilized polymer on the surface of the MWCNTs started to decompose, and weight loss was observed. An increase in the temperature above 470°C did not have any effect on the weight, and the curve was flat. Comparison thermogravimetric curves of the MWCNTs and MIPCNTs showed that approximately 50% of the total weight was related to the MIP content of the MIPCNT. In this figure, it can be seen that the decomposition temperature and weight lost of the MIPs were lower and higher than those of MIPCNT, respectively; this indicated the greater thermal stability of the MIPCNTs due to the immobilization of MIPs on the MWCNT surface.

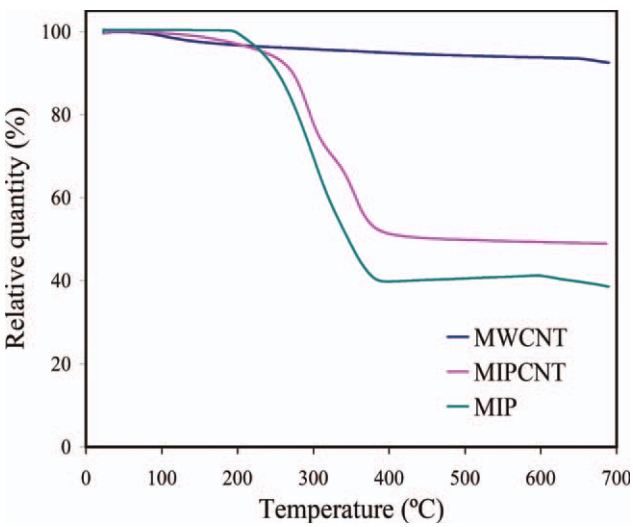

Figure 5 TGA curves of the MWCNTs, MIPCNT, and MIP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

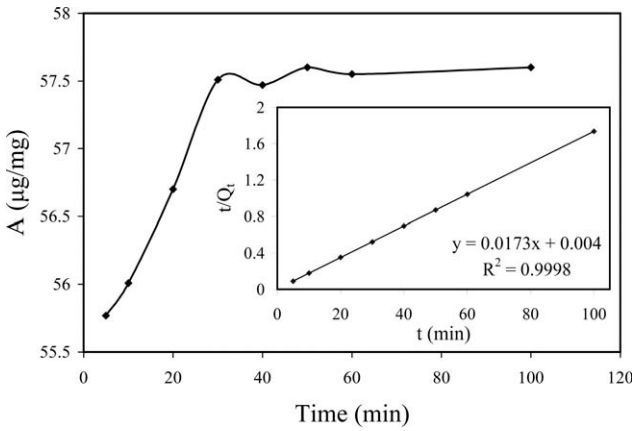

Figure 6 Adsorption kinetics of HCT on the imprinted polymer at a 30.0 μg/mL concentration of HCT. The inset shows the fitting plot with a second-order rate equation.

Adsorption kinetics

Figure 6 shows the dynamic curve of the adsorption of HCT onto the MIPCNTs at a 30.0 μg/mL concentration of HCT. The adsorption capacity of HCT increased with time and reached an almost saturated state after 30 min. The results show that the rapid adsorption of HCT occurred onto the recognition sites of the MIPCNTs. When the imprinted sites on the MIPCNT were occupied, the adsorption rate of HCT decreased. The kinetic data was fitted to linear forms of the first-order rate equation [Eq. (2)] and second-order rate equation [Eq. (3)]:

$$\ln(Q_e - Q_t) = \ln Q_e - k_1 t \tag{2}$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e} \tag{3}$$

where Q_e and Q_t are the adsorption capacities of HCT at equilibrium and at a given time t (μg/mg), respectively, and k_1 (min^{-1}) and k_2 (mg μg^{-1} min^{-1}) are the first-order and the second-order rate constants, respectively. The correlation coefficient (R^2 = 0.9998) of the second-order adsorption model demonstrated a much higher value than that of the first-order model. Also, the calculated equilibrium adsorption capacity ($Q_{e,cal}$ = 59.5 μg/mg) from the second-order model fit well with that of the experimental data ($Q_{e,exp}$ = 57.8 μg/mg). The results reveal that the second-order kinetic equation provided a better estimation in comparison to the first-order kinetic equation.

Evaluation of the selectivity

To evaluate the selectivity, two other drugs, PAP and PM, were selected. The chemical structures of these drugs are shown in Figure 7. It should be mention that MIP is a selective sorbent because, in process formation of MIP, the cavities created in the polymeric network are complementary in size and shape to the template for the subsequent rebinding process. These cavities are able to bind with template. Only molecules that are same in size and conformational structure can trap in the cavity. Therefore, the main problem that should be checked for MIP application is the adsorption of species consisting of functional groups at the surface of the polymer and NIPs. Generally, in most works, other species, such as PAP and PM, that have different functional groups are used to check the adsorption ability of the polymer. The amounts of these drugs that adsorbed on the MIPCNT particles were measured spectrophotometrically at wavelengths of 430 and 255 nm for PAP and PM, respectively, and compared with those on the NIPCNTs. The results show (Fig. 8) that the affinity and specificity of the MIPCNTs for HCT compared to the NIPCNT were greater than those of other examined drugs (5.82-fold). Also, the results show that adsorption of these drugs showed no significant difference between the MIPCNTs and NIPCNTs. This fact could have been due to the high selectivity of the prepared MIPCNTs

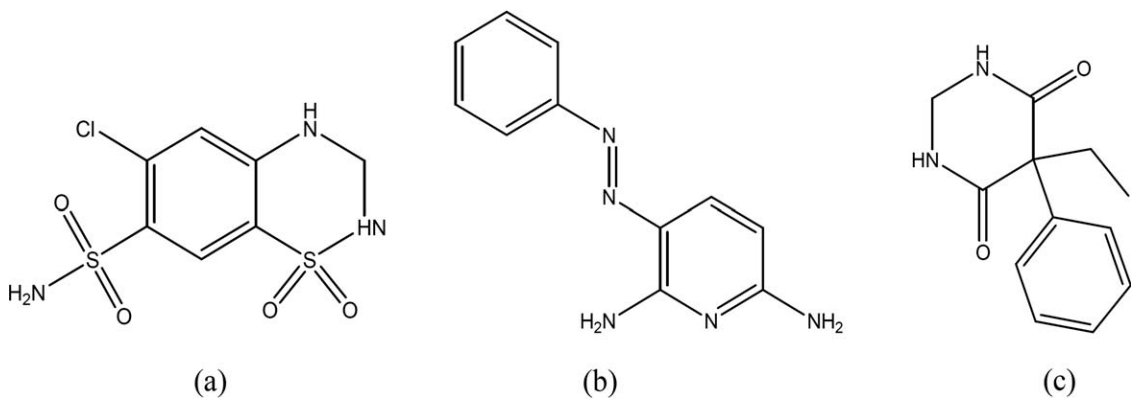

Figure 7 Chemical structures of (a) HCT, (b) PAP, and (c) PM.

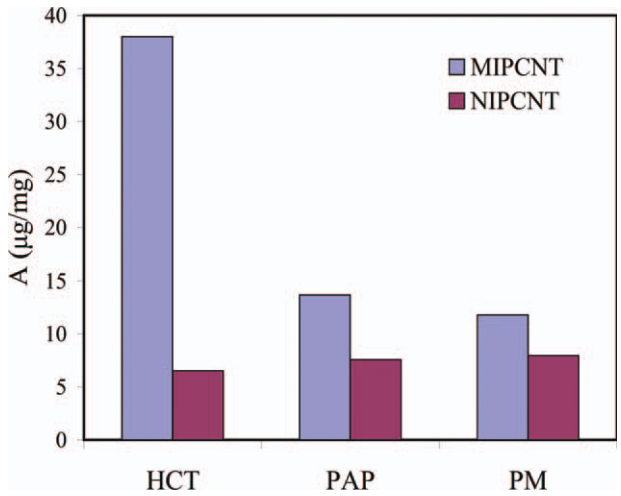

Figure 8 Evaluation of the selectivity of the MIPCNT compared with the NIPCNT for HCT, PAP, and PM at wavelengths of 270, 430, and 255 nm, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for HCT. Essentially, there were no significant differences between MIPCNT and NIPCNT in the adsorption of PAP and PM because they did not have a suitable structure to match the cavities that were created at the polymeric network. However, only the template (HCT) could fit at the cavity, and significant adsorption was observed for the MIPCNTs and NIPCNTs.

CONCLUSIONS

The results of the high A of MIPCNT particles for HCT adsorption suggest that the nanolayer MIP preparation on the surface of the MWCNTs could improve the porous site availability compared to bulk polymerization.[20] Furthermore, some techniques confirmed the homogeneous formation of MIPCNT binding sites; these techniques included SEM, FTIR spectroscopy, and TGA. To decrease the MIP layer thickness on the surface of MWCNTs, the amounts of monomer and crosslinker could be varied. The MIPCNT particles showed a high selectivity for HCT against PM and PAP with same functional groups or size but with different conformations. From the adsorption isotherms, the MIPCNT particles adsorbed more HCT than the NIPCNT particles. The adsorption capacity for HCT was 93.0 μg/mg, and a good imprinting efficiency was achieved (8.1). The adsorption kinetics of HCT on MIPCNT were in agreement with the second-order equation, and $Q_{e,\mathrm{cal}}$ from the second-order model fitted well with $Q_{e,\mathrm{exp}}$.

References

1. Lanza, F.; Sellergren, B. Chromatographia 2001, 53, 599.
2. Andersson, L. I. J Chromatogr B 2000, 739, 163.
3. Dopico-García, M. S.; Cela-Pérez, C.; López-Vilariño, J. M.; González-Rodríguez, M. V.; Barral-Losada, L. F. J Appl Polym Sci 2011, 119, 2866.
4. Chen, Y.; Kele, M.; Sajonz, P.; Sellergren, B.; Guiochon, G. Anal Chem 1999, 71, 928.
5. Xu, L.; Lv, R.; Wang, Y.; Gao, J. J Appl Polym Sci 2010, 118, 881.
6. Mayes, A. G.; Mosbach, K. Anal Chem 1996, 68, 3769.
7. Kempe, M.; Mosbach, K. J Chromatogr A 1995, 691, 317.
8. Bossi, A.; Piletsky, S. A.; Piletska, E. V.; Righetti, P. G.; Turner A. P. F. Anal Chem 2001, 73, 5281.
9. Bossi, A.; Bonini, F.; Turner, A. P. F.; Piletsky, S. A. Biosens Bioelectron 2007, 22, 1131.
10. Bonini, F.; Piletsky, S.; Turner, A. P. F.; Speghini, A.; Bossi, A. Biosens Bioelectron 2007, 22, 2322.
11. Bereli, N.; Andac, M.; Baydemir, G.; Say, R.; Galaev, I. Y.; Denizli, A. J Chromatogr A 2008, 1190, 18.
12. Zhu, X.; Yang, J.; Su, Q.; Cai, J.; Gao, Y. J Chromatogr A 2005, 1092, 161.
13. Puoci, F.; Cirillo, G.; Curcio, M.; Iemma, F.; Spizzirri, U. G.; Picci, N. Anal Chim Acta 2007, 593, 164.
14. Jafari, M. T.; Rezaei, B.; Zaker, B. Anal Chem 2009, 81, 3585.
15. Iijima, S. Nature 1991 354, 56.
16. So, H. M.; Park, D. W.; Jeon, E. K.; Kim, Y. H.; Kim, B. S.; Lee, C. K.; Choi, S. Y.; Kim, S. C.; Chang, H.; Lee, J. O. Small 2008, 4, 197.
17. So, H. M.; Won, K.; Kim, Y. H.; Kim, B. K.; Ryu, B. H.; Na, P. S.; Kim, H.; Lee, J. O. J Am Chem Soc 2005, 127, 11906.
18. Zhang, Z.; Hu, Y.; Zhang, H.; Yao. S. J Colloid Interface Sci 2010, 344, 158.
19. Kan, X.; Zhao, Y.; Geng, Z.; Wang, Z.; Zhu, J. J Phys Chem C 2008, 112, 4849.
20. Rezaei, B.; Mallakpour, S.; Rahmanian, O. J Iran Chem Soc 2010, 7, 1004.